Remarks

Rejections under 35 U.S.C. 112

Claim 39 was rejected under 35 U.S.C. 112 as containing subject matter which was not described in the specification. This rejection is respectfully traversed. The support for each portion of claim 39 is noted below:

39. A method for determining the relative ratio of LDL to HDL in a biological sample comprising

determining the amount of LDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein (see example 7, pages 60-62 for anti-LDL);

determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein (see example 8, pages 62-63); and

determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein (see example 9, pages 65-66).

The examiner has taken the position that example 8 does not provide support for the limitation that the antibodies which bind HDL or LDL do not cross-react with LDL or HDL, respectively. Example 7 shows binding of LDL using mAB

HB_3cB_3. Example 8 shows binding of HDL by mAb $AIbD_5$. Page 17, lines 22-23, state "Mabs that bind to a single apolipoprotein with no significant detectable crossreactivity with other apolipoproteins are considered specific". Page 17, line 24, to page 18, line 16, describes how to determine if there is crossreactivity. Page 27, line 33 to page 28, line 10, states that mAB HB_3cB_3 is specific for LDL and not cross-reactive with other lipoproteins. Page 28, lines 18-25, Tables 1 and 2, state that mAb $AIbD_5$ is specific to HDL.

Therefore the claim is fully supported by the specification.

Claims 1-12, 40, 41, 43, 45, and 47 were rejected under 35 U.S.C. 112 as indefinite. This rejection is respectfully traversed if applied to the amended claims.

Claims 1-11 have been amended to recite specifically that the first and second antibodies must bind different lipoproteins or apolipoproteins, as suggested by the examiner.

Claim 40 step b has been amended to more clearly recite what complex is formed and isolated.

Claim 41, step b, has been amended to delete the reference to the anti-Apo B antibodies which does not appear to belong in the claim.

Claim 42 has been amended to provide antecedent basis for claim 43. Claim 45 has been amended to correct antecedent basis.

Claims 1-13 and 39-43 were rejected under 35 U.S.C. 112, first paragraph, on the basis that the language know refers to binding to the lipoprotein generically, rather than to an epitope. The claims have been amended to more clearly recite "a

monoclonal antibody that binds to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein".

Claim 1 has been amended to delete the language from the preamble relating to the ratio of LDL to HDL since the examiner is correct that VLDL is neither.

Claims 4 and 5 have been amended to delete the reference to "comprise" and instead to refer to either the first or second monoclonal antibodies.

Claim 9 has been amended to correct the tenses. Claim 9 as amended is believed to be clear: the antigen is bound to one antibody, and then bound to a second antibody to a different epitope of the same antigen, the second antibody having stain coupled thereto, to provide for quantitation of the amount of antibody.

Claim 12 has been amended to correctly recite "or" rather than "and" the first and second monoclonal antibodies.

Claim 40 has been amended to more clearly state

Rejections under 35 U.S.C. 102 and 103

Claims 46 and 47 were rejected under 35 U.S.C. 102(b) as disclosed by U.S. Patent No. 4,677,057 to Curtiss, et al. Claims 1, 10 and 11 were rejected under 35 U.S.C. 103 as obvious over U.S. Patent No. 5,126,276 to Fish, et al., in combination with EP 0262854 to Scripps and Forster, et al., Biochem. Soc. Trans. 18(6):1180(1990) Zhow, et al., Hubi Yixueyuan Xueabo. II(4), 298-302 (1998) and Koren, et al. Atheroscloerosis 95, 157-170 (1992), alone or in combination with U.S. Patent No. 4,677,057 to Curtiss. Claim 6 was rejected under 103 as obvious over

Fish in combination with Scripps, Forster, and Zhow, and Koren, et al.. Claims 7 and 8 were rejected under 103 as obvious over Fish, Scripps, Forster, and Zhow, Koren and further in combination with Mills, et al. Laboratory Techniques in biochemistry and molecular biology, vol. 14, pages 472-478 (1984). Claims 12 and 1 were rejected under 103 as obvious over Fish, Scripps, Forster, Zhow, Koren and EO 0 257 778 by Scripps. Claims 42-45 were rejected under 35 U.S.C. 103 as obvious over Koren, et al., (1992). These rejections are respectfully traversed.

Rejections of claims 46 and 47 over Curtiss

Solely to facilitate prosecution of this application, claim 46 and 47 were amended to require the anti-Apo AI antibody to bind to a stable, conformation and lipid indepent epitope. It is still believed that Curtiss clearly fails to disclose such antibodies. As described at col. 13, lines 5-66, Curtiss demonstrates that binding of each of the four antibodies (three anti-Apo I, and one anti-Apo II) were affected by either conformation and/or lipid content of the lipoprotein. See also col. 14, lines 58-68, which further demonstrates that Curtis did NOT expect to produce antibodies which bound in a conformation, lipid-independent manner.

Rejections of claims 1, 10 and 11 over Fish, Scripps, Forster, Zhow, Koren and Curtiss

Claims 1, 10 and 11 define a method for determining the relative ratio of LDL to HDL or at least two different apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second monoclonal antibody molecules

immunoreactive with LDL, HDL or VLDL or at least two different apolipoproteins, wherein the first and second monoclonal antibodies bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, wherein the lipoproteins are LDL, HDL or VLDL;

allowing the monoclonal antibody molecules time to bind to the LDL, HDL or VLDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized monoclonal antibody molecules;

determining the amount of LDL, HDL or VLDL lipoprotein or at least two different apolipoproteins bound by the immobilized monoclonal antibody molecules, and

comparing the amount bound which is specific for LDL, HDL or VLDL or each apolipoprotein in order to calculate the relative amounts of LDL, HDL or VLDL or apolipoproteins.

Fish shows substrates suitable for use of immobilized antibodies that can be dipped into a sample solution to bind to antigen. There is no disclosure of using **multiple** antibodies immunoreactive with LDL, HDL or VLDL on the **same** substrate to provide a comparative ratio. Therefore Fish does not disclose the claimed method. The remaining art does not make up for this deficiency.

Scripps describes assays in which the binding of Apo-B100 relative to the amount of ApoAI is compared. Scripps notes that Apo B-100 is found in LDL and

that Apo AI is found in HDL (page 3, lines 1-6 and lines 28-30). At page 4, lines 48-51, the authors also note that Apo AI is found in **LDL**. Therefore, there is no disclosure of antibodies which are reactive only with LDL OR HDL OR VLDL, and as a result, one could not immobilize these antibodies on the same substrate and obtain comparative data. There is also no disclosure of antibodies which bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein. As described at page 13, lines 10-14, the antibodies to Apo B100 reacted with **all three lipoproteins,** LDL, VLDL and IDL. As described at page 17, lines 10-20, binding of lipid (as in chylomicrons) affects binding to Apo B100. The data at page 18, lines 12-18, indicates that binding of the anti-Apo AI antibodies is affected by lipid, therefore they are not reactive with stable, conformation independent epitopes uninfluenced by lipid content. In contrast, plasma or serum samples can be used undiluted with the conformation and lipid independent antibodies described by applicant. Therefore the antibodies of claim 1 and claims dependent thereon are not disclosed by Scripps. The assay utilized by Scripps (see page 8, lines 1-26) is very complex as a result of the problems that arise as a result. As the examiner is aware from the prosecution of applicants' related applications, it was applicants' development of a technique to produce antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein that was critical to development of the claimed assays and kits for use therein.

Forster describes the desirability of a two antibody assay to obtain the ratio of HDL to LDL but provides no details as to how such an assay could be done or what antibodies are used.

Zhow merely demonstrates that ratios of Apo AI to Apo B may be useful in the diagnosis of heart disease.

Assuming one were motivated to determine the ratio of Apo AI to Apo B, one would still not have the claimed assay. The prior art antibodies fail to completely distinguish between VLDL, LDL and HDL, or the individual apolipoproteins under conditions of varying lipid concentration or conformation. The claims have been amended to clarify and more clearly define these difference.

As noted on page 29, lines 3-13, Koren (1992) describes antibodies to Apo CIII and Apo E. These are not described as antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, and do not completely distinguish between lipoproteins (note the use of the modifying term "predominantly"). Even in combination with the other prior art, one would not achieve the claimed assays.

Curtiss describes four different monoclonal antibodies. These do not bind the same epitopes, but they also do not bind to the epitopes equally well in different lipoprotein populations. Curtiss even states at col. 14, lines 58-60, "There is no reason to assume that conformational variation will be identical for lipid-free and lipid-associated apo-A-I." Curtiss therefore recognizes the problem (the use of

antibodies in an assay is affected by the conformation and lipid content of the lipoprotein) but chooses another approach to solve it. There is nothing that would lead one to conclude that the solution would be to obtain antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, much less how to go about achieving such a goal.

Claim 6 is not obvious from Fish, Scripps, Forster, Zhow, Koren and Luca

The other references are discussed above. Claim 6 adds the further element that the lipid bound to the immobilized antibodies is stained with a lipid stain.

Lucas does not make up for the failure of the other art to provide antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein s deficiency. Lucas also recognizes the desirability of determining the relative amounts of HDL and LDL, as well as apolipoproteins. The Lucas assay requires immobilization of the intact lipoproteins so that the other sample components can be removed. Lucas says (col. 10, lines 9-12) that monoclonals are better for recognition of specific epitopes. The need to remove other sample components clearly indicates that the other sample components would have an effect on the assay as Lucas envisions it, so the antibodies cannot be antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein,

apolipoprotein or lipid associated with the specific lipoprotein. Moreover, based on the disclosure at col. 16, lines 51-60, the immunogens are not treated to delipidate and solubilize and reduce the molecules so that one could obtain antibodies that are reactive with lipid and conformation independent epitopes.

Claims 7 and 8 are not obvious over Fish, Scripps, Forster, Zhow, Koren, Luca and Mills

The other prior art is discussed above. Claim 7 requires staining with specific dyes. Claim 8 requires staining before immersion of the immobilized antibody into the sample.

Mills does teach staining of lipids. However, as discussed above, this would still not lead those skilled in the art to the claimed methods and kits for use therein. The prior art simply fails to provide a means whereby one can place different antibodies on the same substrate to differentiate, measure and compare two different lipoproteins at the same time.

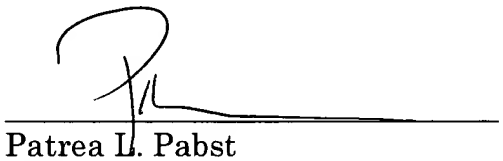
Claims 12 and 13 are not obvious over Fish, Scripps, Forster, Zhow, Koren and Scripps

The art has been discussed above. The examiner's position is that the combination differs by not combining antibody with the sample prior to immersion of the substrate containing the immobilized antibody into the sample. However, this goes to the very heart of the difference between the prior art and what is claimed: it is the differences in the specificity of the antibodies that allows one to differentiate between lipoproteins and therefore to use a single substrate having

antibodies immobilized thereon to detect multiple different antigens, and the prior art fails to teach such antibodies: antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, as claimed.

Allowance of claims 1-13 and claims 39-47 is earnestly solicited. All claims as pending upon entry of this amendment are attached in an Appendix to facilitate review by the Examiner.

Respectfully submitted,



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CERTIFICATE OF MAILING (37 CFR 1.8a)

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Date: September 5, 2001

Jean Hicks

APPENDIX: Marked up Copy of Claims as Amended Upon Entry of the Amendment

1. (three times amended) [The] A method for determining the relative ratio of [LDL to HDL or] at least two different apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second monoclonal antibody molecules* immunoreactive with LDL, HDL or VLDL or at least two different apolipoproteins, wherein the first and second monoclonal antibodies bind to [either] different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, wherein the lipoproteins are selected from the group consisting of LDL, HDL [or] and VLDL [or to different apolipoproteins in a conformation and lipid content independent manner];

allowing the monoclonal antibody molecules time to bind to the LDL, HDL or VLDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized monoclonal antibody molecules;

determining the amount of LDL, HDL or VLDL lipoprotein or at least two different apolipoproteins bound by the immobilized monoclonal antibody molecules, and

comparing the amount bound which is specific for LDL, HDL or VLDL or each apolipoprotein in order to calculate the relative amounts of LDL, HDL or VLDL or apolipoproteins.

2. (amended) The method of claim 1 wherein the antibody molecules immobilized on the solid phase material are immunoreactive with lipoproteins selected from the group consisting of HDL and LDL.

3. (twice amended) The method of claim 2 wherein the antibodies to the HDL or LDL are selected from the group consisting of recombinant antibodies and antibody fragments.

4. (twice amended) The method of claim 3, wherein the <u>first or second monoclonal</u> antibodies [comprise] <u>are</u> the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

5. (twice amended) The method of claim 3, wherein the <u>first or second monoclonal</u> antibodies [comprise] <u>are</u> recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

6. (twice amended) The method of claim 1 further comprising determining the amount of lipoprotein or lipid associating with apolipoprotein by staining of the material bound to the immobilized antibody using a lipid stain.

7. The method of claim 6 wherein the lipid stain is selected from the group consisting of Sudan Red 7B, Oil Red O, and Sudan Black B.

8. The method of claim 6 wherein the lipoprotein lipid is stained prior to immersing the immobilized antibodies.

9. (three times amended) The method of claim 6 further comprising measuring the amount of apolipoprotein or protein associated with lipid in the sample, further comprising the step of providing [antibody] antibodies immunoreactive with the [apolipoproteins] apolipoprotein, wherein the antibodies are coupled to a protein stain, and staining the apolipoprotein or protein associated with lipid in the sample by reacting the protein stain coupled antibodies with the apolipoprotein or protein associated lipid in the sample.

10. The method of claim 1, wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11. The method of claim 1, wherein the biological sample is selected from the group consisting of blood, plasma, and serum.

12. (three times amended) A method of determining the relative concentration of at least two different apolipoproteins in a biological sample comprising:

mixing in solution a first and second monoclonal antibody molecules each immunoreactive with a specific different apolipoprotein into the sample, wherein at least one of the first and second monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of a lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of different apolipoproteins in a conformation and lipid content independent manner;

allowing the monoclonal antibody molecules to bind to the apolipoprotein in the sample,

immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of one of the apolipoproteins,

allowing the third immobilized monoclonal antibody molecules to bind to <u>one of</u> the [apolipoprotein] <u>apolipoproteins bound by either the first or second monoclonal antibodies</u>,

detecting the presence of the apolipoprotein bound by [both one of] <u>either</u> the first [and] <u>or</u> second monoclonal antibodies and the third immobilized monoclonal antibodies, and

determining the amount of apolipoprotein bound by [both one of] <u>either</u> the first [and] <u>or</u> second monoclonal antibodies and the third immobilized monoclonal antibodies.

13. (amended) The method of claim 12 wherein the apolipoprotein bound by one of the monoclonal antibodies in solution is apolipoprotein Apo B-100.

39. (twice amended) A method for determining the relative ratio of LDL to HDL in a biological sample comprising

(a) determining the amount of LDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

(b) determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

(c) determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein, wherein at least one of the monoclonal antibodies to LDL and HDL bind [in a conformation and lipid content independent manner] a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein.

40. (twice amended) A method for determining the relative ratio of VLDL to HDL in a biological sample comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the anti-ApoC-III antibody reactive with Apo C-III with the biological sample to form complexes between the anti-ApoC-III antibody and the Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample containing the anti-ApoC-III antibody bound to the Apo C-III containing lipoprotein particles,

separating the complexed Pan B-anti-ApoC-III antibody-lipoprotein particles from the biological sample, which is the amount of Apo C-III present in VLDL in the anti-Apo C-III anti-Apo B complexed material in the sample; and

(b) determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody specifically immunoreactive with Apo A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the anti-Apo C-III antibody and the Apo C-III containing lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample to form complexes with the anti-Apo C-III antibody-Apo C-III containing lipoprotein particles,

separating the complexed [antibody-] anti-Apo C-III antibody-Apo C-III containing lipoprotein particles from the biological sample,

determining the amount of Apo C-III present in HDL in the anti-Apo C-III-anti-Apo A-I complexed material in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample [and] to Apo C-III present in HDL in the sample, which is the ratio of VLDL to HDL,

wherein the VLDL and HDL are measured in the same sample using immobilized anti-Apo A-I and anti-Apo B or anti-Apo C-III antibodies or measured

by immunoprecipitation with the anti-Apo A-I and anti-ApoB antibodies or anti-Apo C-III antibodies in separate samples,

wherein at least one of the monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo AI, Apo B, [or] and Apo CIII [in a conformation and lipid content independent manner].

41. (twice amended) A method for determining the relative ratio of VLDL to HDL comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody which specifically binds to Apo E associated with VLDL,

contacting the antibodies reactive with Apo E associated with VLDL with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample containing the complexes between the anti-ApoE antibodies and ApoE containing particles to form complexes of anti-ApoB-anti-ApoE-ApoE containing particles, and

determining the amount of Apo E in the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, which is the Apo E present in VLDL in the sample;

(b) removing the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, either by binding of the anti-Apo E antibodies to an immobilized surface or centrifugation of sample to remove the complexes of anti-ApoB-anti-ApoE-ApoE containing particles;

and

(c) determining the amount of HDL in the sample by

determining the amount of Apo E present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I,

[providing monoclonal antibody which binds to Apo E associated with HDL,]

contacting the antibodies reactive with Apo E with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting [Pan B] the Apo A-I monoclonal antibody with the biological sample [for] to form complexes of the anti-ApoE antibodies-ApoE containing particles-anti-[ApoB] ApoA-I,

determining the amount of Apo E present in HDL in the complexes of the anti-ApoE antibodies-ApoE containing particles-anti-[ApoB] Apo A-I in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL,

wherein at least one of the monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo B, Apo AI, [or] and Apo E [in a conformation and lipid content independent manner].

42. (twice amended) A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal or recombinant antibody specifically immunoreactive with Apo C-III, and

monoclonal or recombinant Apo A-I antibody specifically immunoreactive with Apo A-I,

wherein at least one of the monoclonal or recombinant antibodies specifically bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo B, Apo AI, [or] and Apo CIII [in a conformation and lipid content independent manner].

43. (amended) The kit of claim 42 wherein the anti-Apo C-III or anti-A-1 monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent

epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

44. (twice amended) A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody which predominantly binds to Apo E associated with VLDL ,

monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I, and

monoclonal antibody which predominantly binds to Apo E in HDL,

wherein at least one of the antibodies binds to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein.

45. (twice amended) The kit of claim 44 wherein the anti-Apo E or anti-Apo A-I monoclonal [or recombinant] antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments [that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein].

46. (amended) A kit for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

monoclonal or recombinant Apo-A-I antibody specifically immunoreactive with Apo A-I lipoproteins in human plasma; and

monoclonal or recombinant Apo A-II antibody specifically immunoreactive with Apo A-II,

wherein the anti-Apo A-I or anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

47. (amended) The kit of claim 46 wherein the anti-Apo A-I and anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

APPENDIX: Clean Copy of Claims as Pending upon entry of Amendment

1. (three times amended) A method for determining the relative ratio of at least two different apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second monoclonal antibody molecules immunoreactive with LDL, HDL or VLDL or at least two different apolipoproteins, wherein the first and second monoclonal antibodies bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, wherein the lipoproteins are selected from the group consisting of LDL, HDL and VLDL;

allowing the monoclonal antibody molecules time to bind to the LDL, HDL or VLDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized monoclonal antibody molecules;

determining the amount of LDL, HDL or VLDL lipoprotein or at least two different apolipoproteins bound by the immobilized monoclonal antibody molecules, and

comparing the amount bound which is specific for LDL, HDL or VLDL or each apolipoprotein in order to calculate the relative amounts of LDL, HDL or VLDL or apolipoproteins.

2. (amended) The method of claim 1 wherein the antibody molecules immobilized on the solid phase material are immunoreactive with lipoproteins selected from the group consisting of HDL and LDL.

3. (twice amended) The method of claim 2 wherein the antibodies to the HDL or LDL are selected from the group consisting of recombinant antibodies and antibody fragments.

4. (twice amended) The method of claim 3, wherein the first or second monoclonal antibodies are the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

5. (twice amended) The method of claim 3, wherein the first or second monoclonal antibodies are recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

6. (twice amended) The method of claim 1 further comprising determining the amount of lipoprotein or lipid associating with apolipoprotein by staining of the material bound to the immobilized antibody using a lipid stain.

7. The method of claim 6 wherein the lipid stain is selected from the group consisting of Sudan Red 7B, Oil Red O, and Sudan Black B.

8. The method of claim 6 wherein the lipoprotein lipid is stained prior to immersing the immobilized antibodies.

9. (three times amended) The method of claim 6 further comprising measuring the amount of apolipoprotein or protein associated with lipid in the sample, further comprising the step of providing antibodies immunoreactive with

the [apolipoproteins] apolipoprotein, wherein the antibodies are coupled to a protein stain, and staining the apolipoprotein or protein associated with lipid in the sample by reacting the protein stain coupled antibodies with the apolipoprotein or protein associated lipid in the sample.

10. The method of claim 1, wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11. The method of claim 1, wherein the biological sample is selected from the group consisting of blood, plasma, and serum.

12. (three times amended) A method of determining the relative concentration of at least two different apolipoproteins in a biological sample comprising:

mixing in solution a first and second monoclonal antibody molecules each immunoreactive with a specific different apolipoprotein into the sample, wherein at least one of the first and second monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of a lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of different apolipoproteins in a conformation and lipid content independent manner;

allowing the monoclonal antibody molecules to bind to the apolipoprotein in the sample,

immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of one of the apolipoproteins,

allowing the third immobilized monoclonal antibody molecules to bind to one of the apolipoproteins bound by either the first or second monoclonal antibodies,

detecting the presence of the apolipoprotein bound by either the first or second monoclonal antibodies and the third immobilized monoclonal antibodies, and

determining the amount of apolipoprotein bound by either the first or second monoclonal antibodies and the third immobilized monoclonal antibodies.

13. (amended) The method of claim 12 wherein the apolipoprotein bound by one of the monoclonal antibodies in solution is apolipoprotein Apo B-100.

39. (twice amended) A method for determining the relative ratio of LDL to HDL in a biological sample comprising

(a) determining the amount of LDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

(b) determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

(c) determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein, wherein at least one of the monoclonal antibodies to LDL and HDL bind a stable, conformation independent epitope that is

uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein.

40. (twice amended) A method for determining the relative ratio of VLDL to HDL in a biological sample comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the anti-ApoC-III antibody reactive with Apo C-III with the biological sample to form complexes between the anti-ApoC-III antibody and the Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample containing the anti-ApoC-III antibody bound to the Apo C-III containing lipoprotein particles,

separating the complexed Pan B-anti-ApoC-III antibody-lipoprotein particles from the biological sample, which is the amount of Apo C-III present in VLDL in the anti-Apo C-III anti-Apo B complexed material in the sample; and

(b) determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody specifically immunoreactive with Apo A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the anti-Apo C-III antibody and the Apo C-III containing lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample to form complexes with the anti-Apo C-III antibody-Apo C-III containing lipoprotein particles,

separating the complexed anti-Apo C-III antibody-Apo C-III containing lipoprotein particles from the biological sample,

determining the amount of Apo C-III present in HDL in the anti-Apo C-III-anti-Apo A-I complexed material in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample to Apo C-III present in HDL in the sample, which is the ratio of VLDL to HDL,

wherein the VLDL and HDL are measured in the same sample using immobilized anti-Apo A-I and anti-Apo B or anti-Apo C-III antibodies or measured by immunoprecipitation with the anti-Apo A-I and anti-ApoB antibodies or anti-Apo C-III antibodies in separate samples,

wherein at least one of the monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo AI, Apo B, and Apo CIII.

41. (twice amended) A method for determining the relative ratio of VLDL to HDL comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody which specifically binds to Apo E associated with VLDL,

contacting the antibodies reactive with Apo E associated with VLDL with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample containing the complexes between the anti-ApoE antibodies and ApoE containing particles to form complexes of anti-ApoB-anti-ApoE-ApoE containing particles, and

determining the amount of Apo E in the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, which is the Apo E present in VLDL in the sample;

(b) removing the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, either by binding of the anti-Apo E antibodies to an immobilized surface or centrifugation of sample to remove the complexes of anti-ApoB-anti-ApoE-ApoE containing particles;

and

(c) determining the amount of HDL in the sample by

determining the amount of Apo E present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I,

contacting the antibodies reactive with Apo E with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting the Apo A-I monoclonal antibody with the biological sample to form complexes of the anti-ApoE antibodies-ApoE containing particles-anti-ApoA-I,

determining the amount of Apo E present in HDL in the complexes of the anti-ApoE antibodies-ApoE containing particles-anti-Apo A-I in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL,

wherein at least one of the monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo B, Apo AI, and Apo E.

42. (twice amended) A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal or recombinant antibody specifically immunoreactive with Apo C-III, and

monoclonal or recombinant Apo A-I antibody specifically immunoreactive with Apo A-I,

wherein at least one of the monoclonal or recombinant antibodies specifically bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo B, Apo AI, and Apo CIII.

43. (amended) The kit of claim 42 wherein the anti-Apo C-III or anti-A-1 monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

44. (twice amended) A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody which predominantly binds to Apo E associated with VLDL ,

monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I, and

monoclonal antibody which predominantly binds to Apo E in HDL,

wherein at least one of the antibodies binds to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein.

45. (twice amended) The kit of claim 44 wherein the anti-Apo E or anti-Apo A-I monoclonal antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments.

46. (amended) A kit for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

monoclonal or recombinant Apo-A-I antibody specifically immunoreactive with Apo A-I lipoproteins in human plasma; and

monoclonal or recombinant Apo A-II antibody specifically immunoreactive with Apo A-II,

wherein the anti-Apo A-I or anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

47. (amended) The kit of claim 46 wherein the anti-Apo A-I and anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.